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Partial Reduction of a Humic Acid

INGVAR LINDQVIST

Department of Chemistry and Molecular Biology

Abstract. The charge-transfer acceptor properties of a partially reduced humic acid have been established by spectrophotometric studies at varying pH of the interactions with 1,2-dihydroxybenzene. More detailed studies have also been made with the original humic acids. It is suggested that the colours of humic acids in the visible range cannot be fully explained by simple quinhydrone interaction or by the presence of semi-quinone groups.

INTRODUCTION

There are many reports about reduction of humic acids with strong reducing agents. A good survey has been given by Schnitzer & Kahn (1972) where results of reduction with zinc dust distillation and with sodium amalgam are presented in detail. In those cases the reduction has been driven to complete formation of low-molecular products. The aim with the present study has been to make a partial reduction of a humic acid. After the reduction the main features of the humic acid should be preserved. The molecular volume should thus not be appreciably changed, nor the acidic properties. The UV and visible spectra could very well be changed but the featureless character should be preserved with the typical monotonous change with wave length. We have also been interested in the charge-transfer properties which in an earlier paper (Lindqvist, 1982) were established for the original humic acid. These properties are particularly interesting because they are probably the cause of the sequestration of aromatic compounds by humic acids. The reducing agent used has been sodium dithionite at pH 8. Similar experiments have been made with fulvic acids by Senesi, Chen & Schnitzer (1977).

EXPERIMENTAL

The soil and the preparation of the humic acid samples and the methods to study the charge-transfer acceptor properties have been described in an earlier paper (Lindqvist, 1982). All experiments were made with aqueous solutions of 1,2-dihydroxybenzene. The increases in absorbance have been found by compensating for the dilution but the effects have also been confirmed by comparison with equal additions of pure buffer.

The gel chromatographic studies were performed at Sephadex G 200. Dialysed, freeze-dried humic acids were dissolved in phosphate buffers (pH 8) and eluted with the same buffer. The reduction was carried out in the following way: 1% humic acid was dissolved in a 0.1 M phosphate buffer (pH 8) which contained 7.5% $Na_2S_2O_4$. The solution was left over night, was then dialysed and finally freeze-dried. In some experiments the reaction was followed directly in the solution.

RESULTS AND DISCUSSION

Spectral changes in the batch reaction

The spectral changes for the batch reaction are given in Fig. 1. They indicate a two-step reaction. Immediately after mixing there is a drastic decrease of absorbance at lower wave lengths (by almost 30% at 520 nm) but an increase at wave lengths over 700 nm (more than 40% at 780 nm). In the next step there is a slow decrease of absorbance at all wave lengths (from 20% at 520 nm to 15% at 780 nm). A further addition of $Na_2S_2O_4$ does not change the absorbance. In an experiment with air bubbling starting after the immediate reaction the slow decrease continued for an hour but was followed by a slow recovery. After 45 hours the absorbance at 780 nm was back at its initial value in the unreduced state and at 520 nm the recovery was about 80%. The reaction is thus not completely reversible with oxygen as the reoxidizing agent. In other experiments persulfate was tried with the same result.

We have also observed that a very small fraction of the reduced humic acid is lost at the dialysis as a slightly yellow solution. This loss is not large enough to explain the decreased absorbance at

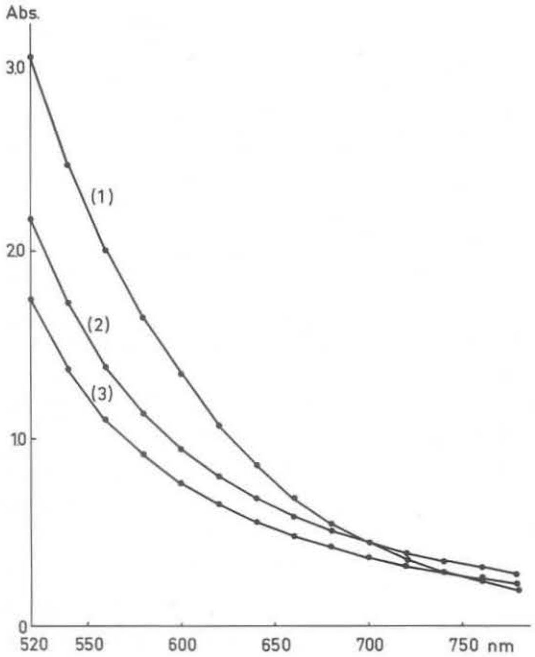

Fig. 1. Spectra of humic acid (1), the same humic acid directly after treatment with sodium dithionite (2) and after 4.5 hours (3).

the lower wave lengths but indicates further complications and might explain the results in the next section.

The dialysed, freeze-dried and redissolved reduced humic acid was studied with respect to spectra, elementary composition, charge-transfer acceptor properties and pH dependence of spectra.

Spectra of the reduced humic acid

The absorbancies in the wave length region 320–580 nm have been measured for a 0.01 % solution of reduced humic acid and compared with those of the unreduced humic acid at the same concentration. The general change of spectra is the same as in the batch experiment but the decrease in absorbance at lower wave lengths is smaller and shifts to an increase already at 520 nm (Fig. 2). Even if the reoxidation probably is larger when the humic acid is taken away from the dithionite solution it is obviously not a case of simple reversibility.

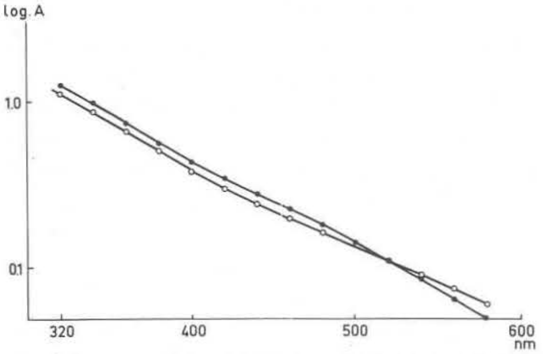

Fig. 2. Spectra of 0.01 % humic acid (●) and 0.01 % reduced humic acid (○).

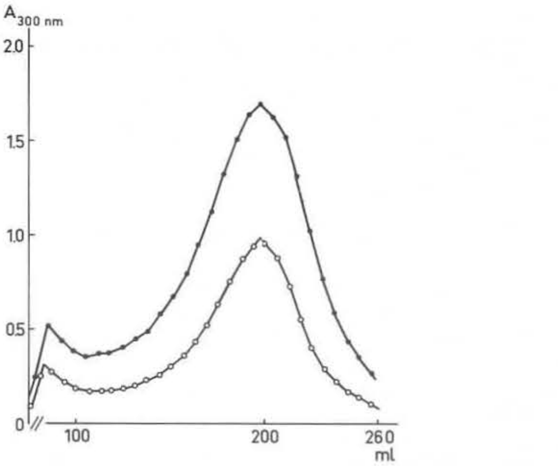

Fig. 3. Gel chromatography on Sephadex G 200 of humic acid (●) and reduced humic acid (○).

The elementary composition of the reduced humic acid

The elementary analyses indicate only very small changes in composition. The C : N and C : O ratios are constant within the limits of error (1 % decrease and 2 % increase, respectively). The only experimentally proven change is the increase in the hydrogen content. The H : C and H : O ratios have increased with 6 and 8 %, respectively. This change in composition could correspond to a reduction of carbonyl or semiquinone groups to hydroxyl groups. The very small change in composition leads, however, to appreciable spectral changes.

Gel chromatography of the reduced humic acid

The absorbancies at 300 nm for the gel chromatography fractions are shown in Fig. 3 for the original and the reduced humic acid. It is obvious that the molecular volumes have not changed because of the reduction. Both maxima (the first at the void volume) are at the same volumes and the relative heights are identical.

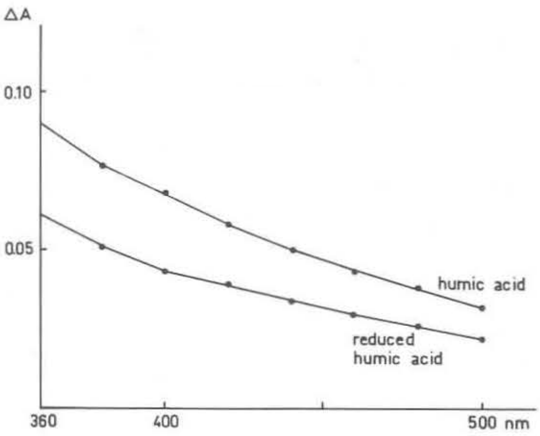

Fig. 4a. Differential spectrum of 2 ml 0.1% humic acid treated with 200 µl 40% 1,2-dihydroxybenzene at pH=3. The figure also shows the differential spectrum of reduced humic acid under the same conditions.

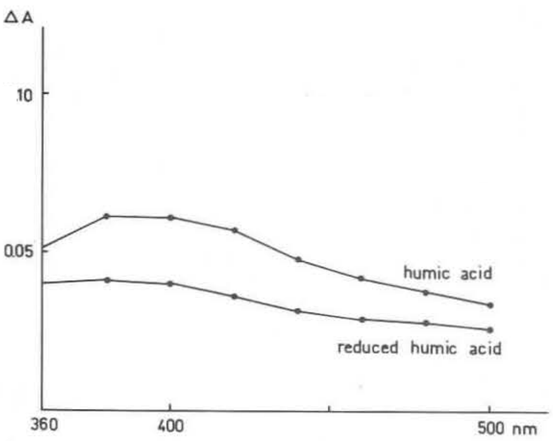

Fig. 4b. Differential spectrum of 2 ml 0.1% humic acid treated with 200 µl 40% 1,2-dihydroxybenzene at pH=5. The figure also shows the differential spectrum of reduced humic acid under the same conditions.

Charge-transfer reactions with donor molecules at varying pH

In the first paper on the charge-transfer properties of humic acids (Lindqvist, 1982) the main purpose was to prove that there existed a charge-transfer interaction and that the charge-transfer spectra had a great similarity in general shape with the spectra of the humic acids. In this paper we have tried to go a little further and make at least some more detailed studies of the effect of reduction and of change in pH. The experimental difficulties are of two types. The charge-transfer spectra are obtained as small differences between two large absorbance values and thus rather uncertain. Furthermore the solutions of 1,2-dihydroxybenzene are very sensitive to oxidation at higher pH. Already at pH 7 there are indications of complications and in this paper only results obtained at pH 3, 4 and 5 will be presented. The results are not averaged but taken from individual experiments in order to show the uncertainties better. The negative charge-transfer effect at high pH reported in the earlier paper was an artifact but the complications at higher pH are large and it was not considered necessary in this context to go further on this problem which, however, will be taken up later.

Two comparisons were made. Original and reduced humic acids were compared on the same weight percentage levels and for both types comparisons were made between different pH values. It is found (Fig. 4*a* and *b*) at all ~~three~~ two pH values studied here that the absorbance increment is smaller for the reduced humic acid in the wave length region 360–500 nm. One would expect a decreasing interaction with donor molecules after the reduction because the acceptor properties should be weakened. The absorbance increments give, however, only a weak indication about the strength of the charge-transfer interaction. At higher wave lenghts the rather uncertain results actually indicate an opposite trend. The conditions are obviously much more complicated than expected for *one* simple charge-transfer interaction. It would be desirable to assess the strength of the interaction by some more direct method.

In the earlier paper it was reported that the charge-transfer effect did not show a maximum but only a shoulder at the long wave lengths. The extension of the studies to lower wave lengths has revealed a small maximum at higher pH than 4 (Fig. 4*b*) which seems to be established within the limits of error of the measurements.

In the earlier paper we dared not claim any variation with pH at longer wave lengths. In the wave length region 320–380 nm, however, there are obvious differences (Fig. 5*a* and *b*) indicating a decreasing effect with pH from 3 to 5 for humic acid and from 3 to 4 for reduced humic acid.

DISCUSSION

There are a number of theoretical questions raised by the results of these experiments and those of an earlier paper (Lindqvist, 1982):

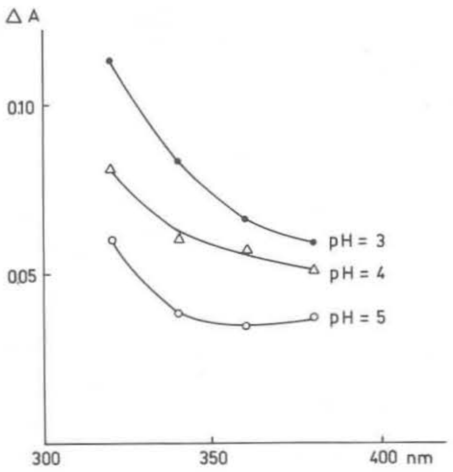

Fig. 5 a. Differential spectra of 2 ml 0.02 % humic acid, treated with 200 μl 40 % 1,2-dihydroxbenzene at three different pH-values.

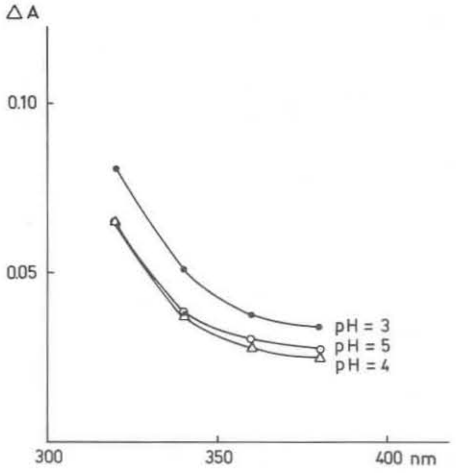

Fig. 5 b. Differential spectra of 2 ml 0.02 % reduced humic acid, treated with 200 μl 40 % 1,2-dihydroxybenzene at three different pH-values.

1. What causes the increase of absorbance found by reaction of humic acids and donor molecules like dihydroxybenzenes? We have assumed that the increased absorbance is the result of complicated charge-transfer interactions and this assumption is corroborated in the present study because reduction is ruled out as a possible cause. Quinone groups could be involved as acceptors in such an interaction but they are in the case only partially reduced because the acceptor properties are not lost by the reduction.

2. What is the cause of the increase in absorbance with increasing pH for the original as well the reduced humic acid? It has been argued, on the basis of EPR studies that the formation of semiquinone anions should be formed at higher pH (Tollin, Reid & Steelink, 1963; Senesi & Schnitzer, 1977). There are, however, also smaller changes at lower pH which must be explained in another way. As has been emphasized earlier (Lindqvist, 1968) the effect is covering a wide range of wave lengths and cannot be explained by the existence of a single chromophore. Further studies are needed.

3. Are the humic acid molecules built up by smaller units kept together by charge-transfer interaction (and hydrogen bonding)? Results by Schnitzer (reported in Schnitzer & Khan, 1972) indicate surprisingly small molecular weights obtained by cryoscopic studies. The suggestion that these units are kept together by hydrogen bonds must be rejected as easily seen if one tries to build the models suggested, which is impossible. A much more probable cause would be charge-transfer interaction which would also explain the sequestration of different aromatic compounds, many of which are environmental polluters. It would also be tempting to explain the appreciable variation in molecular volume with pH in this way (e.g. Lindqvist & Lindqvist, 1979). The reduction does not, however, change the molecular volume appreciably at pH 8 (Fig. 3), a fact which could indicate that the reduction does not reach the inner parts of the humic acid molecule. Further studies are needed to find a more definite answer.

4. Can the absorption spectra of humic acids be explained as due to charge-transfer interaction of quinhydrone type? With quinhydrone type is here meant all types of quinone-phenol interaction. Studies which will be published in a later paper show e.g. that 1,2-dihydroxybenzene gives a complex with p-quinone. In that paper it will also be shown that the absorbance in the visible range for a quinhydrone solution, compared with a humic acid solution of the same concentration is only of the order of 10^{-2}. A direct comparison would require a knowledge of the association of quinhydrone and of the fraction of quinhydrone interactions in humic acids. The former can be roughly estimated for a 0.1 % acid solution from the associa-

tion constant 1.06 (Tsubomura, 1953) in 0.05 M HCl solution to be of the order of 10^{-3}. The latter should be definitely less than 10^{-2}. This leaves us with at least a factor of ten larger absorbance in the humic acid than in quinhydrone which again shows that the spectra cannot be explained only as simple quinhydrone type interaction. An increase can be expected by coupling to an extended delocalization. The semiquinone groups can certainly not be the main factor as shown by the effect of the reduction in this paper. The EPR-signals disappear by reduction with dithionite (Atherton et al., 1967) while the absorbance at longer wave lengths increases. It is very possible, however, that the presence of semiquinone groups can enhance other effects. The fact that the absorbance covers a wide range of wave lengths further proves that it cannot be explained by one chromophore.

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Ingvar Lindqvist
Department of Chemistry
and Molecular Biology
S-750 07 UPPSALA, Sweden